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HARRIET M. STRIMPEL; NEW ENGLAND BIOLABS, INC.
240 COUNTY ROAD
IPSWICH, MA 01938-2723

EXAMINER
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BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application Number: 10/622,240
Filing Date: July 18, 2003
Appellant(s): TZERTZINIS ET AL.

Harriet M. Strimpel
For Appellant

EXAMINER'S ANSWER

(1) This is in response to the appeal brief filed 08/13/2007 appealing from the Office action mailed on 01/04/2007.

(2) Related Appeals and Interferences

The examiner is not aware of any related appeals, interferences, or judicial proceedings that will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(3) Status of Claims

The statement of the status of claims contained in the brief is correct.

This appeal involves claims 1, 2, 5-7, 9, 12-14, 16-18, 20, and 47.

Claims 1, 7, 13, and 18 been amended subsequent to the final rejection.

Claims 8, 10, 15, 19, and 21-46 have been withdrawn from consideration as not directed to the elected invention and species.

Claims 3, 4, and 11 have been canceled.

(4) Status of Amendments After Final

The appellant's statement of the status of amendments after final rejection contained in the brief is correct.

The amendment after final rejection filed on 06/11/2007 has been entered.

(5) Summary of Claimed Subject Matter

The summary of claimed subject matter contained in the brief is correct.

(6) Grounds of Rejection to be Reviewed on Appeal

The appellant's statement of the grounds of rejection to be reviewed on appeal is correct.

(7) Claims Appendix

The copy of the appealed claims contained in the Appendix to the brief is correct.

(8) Evidence Relied Upon

No evidence is relied upon by the examiner in the rejection of the claims under appeal.

(9) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1, 2, 5-7, 9, 12-14, 16-18, 20, and 47 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Yang et al. (PGPUB 2004/0014113), in view of Gross et al. (Nucleic Acids Research, 1987, 15: 431-442).

Yang et al. teach a method of producing a mixture of 15-30 nucleotides long esiRNAs (i.e., a plurality of double-stranded RNA fragments) by digesting a large double-stranded mRNA with *E. coli* RNase III in the presence of magnesium; the ratio of RNase III to double-stranded RNA varies (it could be 1 μg enzyme to 100 μg double-stranded RNA, i.e., 0.01:1), digestion is complete in less than 6 hours, and the resulting esiRNAs mixture represents a set of overlapping fragments capable of cleaving the target mRNA when introduced into an eukaryotic cell (claims 1, 2, 9, 12) (p. 1, paragraph 0004, p. 2, paragraph 0015, p. 6 paragraphs 0053-0056, p. 7, paragraph 0057, p. 8, paragraph 0079, Fig. 1B). Yang et al. teach isolating their esiRNAs mixture, i.e., they teach a purified set of double-stranded RNA fragments; the fragments represent a substantial portion, or the complete sequence, of the double-stranded mRNA from which they are derived (claims 13, 14, and 47) and at least one or more than 50% of the fragments are capable of cleaving the target mRNA (claims 17 and 18) (p. 5, paragraph 0044, p. 6, paragraphs 0053-0056). Yang et al. also teach reaction conditions that efficiently generate 20-25 nucleotide long esiRNAs, i.e., the method generates 15-30 nucleotides long fragments with a yield of greater than 30% (claim 16) (p. 2, paragraph 0015).

Yang et al. teach a reaction mixture containing magnesium and not manganese, as recited in claims 5-7. Gross et al. teach that *E. coli* RNase III can use manganese for catalytic activity, wherein manganese is used at a concentration of 5mM and wherein the use of manganese enables cleavages at sites that are not exposed in the presence of magnesium (claims 5 and 6) (p. 432 first paragraph, p. 439, first full paragraph, p.

440, Fig. 4, p. 441, explanations for Fig. 4). It would have been obvious to one of skill in the art, at the time the invention was made, to modify the method of Yang et al. by replacing magnesium with manganese, with a reasonable expectation of success. One of skill in the art would have been motivated to do such for a more efficient production of esiRNAs; one of skill in the art would have known that the use of manganese results in a higher cleavage efficiency because Gross et al. teach that replacing magnesium with manganese promotes the cleavage of additional sites in the double-stranded RNAs (p. 432, first paragraph). One of skill in the art would have been expected to have a reasonable expectation of success in using manganese because the art teaches that manganese can be successfully used for the catalytic activity of RNase III.

With respect to the limitations of the ratio enzyme:substrate being 0.25:1 (claim 1) or of the manganese concentration being 10 to 20 mM (claim 7), it would have been obvious to the one of skill in the art to vary the parameters in a given method with the purpose of optimizing the results; it is generally not inventive to discover the optimal working conditions of a prior art method, such conditions can be identified by routine experimentation. The limitation of silencing *in vivo* (claim 20) is not innovative over the prior art, which teaches the *in vivo* use of siRNA.

Thus, the claimed invention was *prima facie* obvious at the time the invention was made.

(10) Response to Argument

It is noted that Applicant does not dispute the teachings of the prior art. However, Applicant argues that the rejection is inappropriate because the claimed invention was not predictable, and therefore, not obvious at the time the invention was made. Applicant submits that the Examiner did not establish a case of *prima facie* obviousness because she did not provide any reason to combine and modify the teachings of the prior art to arrive at the claimed invention and because she failed to rebut Applicant's position that the cited references teach away from the claimed invention. Applicant argues that the claimed invention does not involve the predictable use of prior art elements according to their established functions; the claimed invention works in an unexpected and fruitful way as compared to the prior art.

A. Claim 1

With respect to the rejection of claim 1 over Yang et al., Applicant argues that they teach limited RNase III digestion in an effort to obtain molecules of approximately 15-30 nucleotides long (paragraphs 0015 and 0053) and that this limited digestion results in a smear on the agarose gel because of the wide range of sizes of fragments resulting from the partial digestion (Fig. 1B). Yang et al. then selectively purify the fragments corresponding to 21-30 bp from all the other RNAs resulting from the limited digestion (paragraph 0053). Applicant argues that the differences between Yang et al. and the invention of claim 1 are important: (i) the reaction mixture of Yang et al. does not contain a divalent transition metal cation and Yang et al. are silent about the possibility of including one, (ii) Young et al. do not teach a ratio of enzyme to substrate of about or greater than 0.25:1; Young et al. use a ratio of 0.001:1 (paragraph 0079),

and (iii) Young et al. do not demonstrate the production of overlapping fragments, as required by the claimed invention. Applicant argues that the Examiner did not provide any argument for changing the limited digestion of Yang et al. by increasing the amount of the enzyme by at least 20-fold and by using a divalent metal cation, or that this would lead to a method of producing overlapping double-stranded RNA fragments. Applicant argues that, in order to improve the digestion, Yang et al. used a limited incubation time, reduced the amount of enzyme, and lowered the temperature, without suggesting the need for further improvement of a different character.

With respect to Gross et al., Applicant argues that they us RNase III to cleave a 141 nucleotide long single-stranded RNA having three double-stranded regions, none of which is longer than 15 bp, wherein additional sites are cleaved in the presence of manganese as compared to magnesium (Abstract, Fig. 3, p. 432). Applicant argues that Gross et al. do not relate to the production of overlapping double-stranded RNA fragments of 15-30 nucleotides, do not teach digesting a preparation of large double-stranded RNA, and do not teach using a ratio of 0.25:1. Applicant argues that, even in view of Gross et al., one of skill in the art would not have a reason to modify the method of Yang et al. in such a way as to result in the claimed invention. Applicant argues that, by taking the position that one of skill in the art would want to replace magnesium with manganese to promote the cleavage of additional sites, the Examiner disregards that Yang et al. teach the critically limiting of digestion; Yang et al. teach that exhaustive digestion leads to products of 12-15 bp in length that are unable to trigger and RNAi response (paragraph 0015). Therefore, Applicant argues that one of skill in the art

would not have wanted to promote the cleavage of additional sites in the method of Yang et al., as this would only exacerbate the problem Yang was trying to address by limiting the digestion. Applicant submits that, because Yang et al. teach limiting digestion and Gross et al. teach increasing digestion, the references themselves teach away from their combination and from the claimed invention.

With respect to the claimed ratio of 0.25:1, Applicant argues that the Examiner did no to provide any sound basis for further modifying Yang et al. to increase the amount of enzyme by at least 20-fold. Applicant submits that no rationale has been provided explaining why one of skill in the art would want to increase the amount of enzyme despite of the importance in Yang et al. of limiting digestion. Additionally, Applicant argues, making both modifications, i.e., using manganese and increasing enzyme concentration, would multiply the increase in a cleavage reaction that Yang et al. try to limit.

Regarding the production of overlapping fragments, Applicant points out that, in the Advisory Action of 07/12/2007, the Examiner argues that producing overlapping fragments is an inherent property of RNase III. Applicant argues that Gross et al. teach that RNase III cleaves RNA at three specific sites in the presence of magnesium and at two additional specific sites in the presence of manganese (Abstract, Fig. 3). Thus, Applicant argues, the cited art relied upon shows that RNase III, under some circumstances, specifically cleaves RNA. Applicant argues that the discovery that overlapping fragments could be produced, covering a substantial percentage of a large substrate, was Applicant's discovery and was not evidenced in the art on which the

rejection relies. Applicant submits that, because there was no sound reason to combine and modify the teachings of the prior art to arrive at the claimed invention, no *prima facie* case of obviousness has been established.

Regarding the unpredictability of the invention, Applicant argues that, considering the teachings of Yang et al and Gross et al., one of skill in the art would have predicted that modifying Yang et al. increasing the enzyme concentration and using manganese would rapidly cleave the RNA into fragments too small to be of interest. Applicant however discovered that the claimed invention permits the preparation of overlapping fragments of defined size. Applicant points out that the claimed invention offers significant advantages, such as possibility to generate fragments less than 50 bases without degrading them wherein size fractionation is not required and wherein the fragments represent a substantial portion of the substrate sequence (p. 28, p. 74, line 23, Fig. 4A and 4B).

B. Claims 2 and 9

Applicant argues that the rejection of claims 2 and 9 must be reversed fro all the reasons set forth above with respect to the rejection of claim 1. Additionally, Applicant points out that the invention of claim 2 requires that the plurality of overlapping fragments be the product of a complete digestion. Applicant argues that the references cannot render the invention of claim 2 obvious because Yang et al. teach that exhaustive cleavage leads to products that are unable to trigger RNAi (paragraph 0015). Therefore, Yang et al. teach away from the invention of claim 2.

C. Claims 5-7

Applicant argues that the rejection of claims 5-7 must be reversed fro all the reasons set forth above with respect to the rejection of claim 1. Additionally, because claims 5-7 require manganese, which is the transition metal cation reported by Gross et al. to promote additional cleavage events, and therefore, Applicant asserts that one of skill in the art would not have been motivated to chose manganese. Applicant submits that claims 5-7 cannot be obvious.

D. Claim 12

Applicant argues that the rejection of claim 12 must be reversed fro all the reasons set forth above with respect to the rejection of claim 1. Additionally, Applicant argues that claim 12 recites a method of silencing the expression of a target gene and that, based on the teachings of the art, one of skill in the art would not have reasonably expected that a digestion according to claim 1 would lead to products to be used in a method of silencing expression of a target gene. Accordingly, claim 12 cannot be obvious in view of the cited references.

E. Claims 13, 14, 16-18, 20, and 47

Applicant points out that the invention of claims 13, 14, 16-18, 29, and 47 is drawn to a purified set of overlapping fragments with a size of 15-30 nucleotides, wherein the fragments collectively represent a substantial portion of the large double-stranded RNA from which the fragments are derived. Applicant argues that neither Yang et al. nor Gross et al. suggest a purified set of double-stranded RNA fragments that are overlapping and represent a substantial portion of the RNA from which they are derived. Applicant argues that the Examiner failed to explain why one of skill in the art

would find the claimed invention predictable in view of the references. Applicant submits that Gross et al. suggest, at least in one context, RNase III-mediated cleavage happens at defined positions and, if cleavage site were at fixed positions, one of skill in the art would not expect that cleavage products would be overlapping. Similarly, one of skill in the art would not expect, even if overlapping fragments occasionally occurred, that they would collectively represent a substantial portion of the sequence of the target RNA. Applicant argues that the Examiner has presented no evidence that one of skill in the art would have expected to produce overlapping fragments representing a substantial portion of the sequence of the target RNA.

Applicant concludes that the Examiner failed to provide any evidence that the claimed invention was predictable in view of the art.

It is noted that the above arguments were previously presented and not found persuasive for the following reasons:

A. Claim 1

Applicant points out the important differences between Yang et al. and the invention of claim 1. However, Applicant's arguments are individually directed against Young et al.; one cannot show nonobviousness by attacking the reference individually where the rejection is based on the combination of Young et al. and Gross et al. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

With respect to the argument that limited digestion in Yang et al. results in a smear on the agarose gel because of the wide range of sizes of fragments resulting from the partial digestion (see Yang et al., Fig. AB), it is noted that claim 1 does not require the absence of fragments of different sizes; claim 1 only requires that overlapping fragments of 15-30 bases be produced, which is taught by Yang et al. Additionally, it is noted that smears are obtained only at short incubation times (1 or 3 min), while 15 min incubation time results mainly in fragments that are 15-30 bases long (see Yang et al., paragraph 0053 and Fig. 1B). Therefore, it is clear that Yang et al. teach conditions that result in the accumulation of 15-30 bases long fragments, which do not result in exhaustive digestion. It is noted that Yang et al. only caution against exhaustive digestion (see p. 2, paragraph 0015). By teaching limited digestion, Yang et al. do not necessarily teach that complete digestion to fragments of 15-30 bp cannot be achieved; on the contrary, as noted above, Yang et al. teach conditions of limited digestion (i.e., 15 min at 37°C) wherein fragments of 15-30 bases are produced.

With respect to the argument that Yang et al. do not teach a method that uses manganese, it is noted that it is the combination of Yang et al. and Gross et al. that teaches a method that uses a reaction mixture containing manganese (see above). With respect to the argument that Yang et al. do not teach enzyme:substrate ratio of 0.25:1, while it is true that Young et al. use 1 μg enzyme and 100 μg double-stranded RNA substrate (i.e., a ratio of 0.01:1, and not 0.001 as submitted by Applicant), Gross et al. teach that the catalytic properties of RNase III change when magnesium is substituted with manganese (p. 441 bridging p. 442, p. 442). Therefore, one of skill in

the art, having been aware of this, would have known and be motivated to re-optimize the reaction conditions accordingly, to achieve optimum results; such optimization procedures are routine in the art. There is no evidence on the record that the claimed ratio of 0.25:1 results in unexpected properties, as compared to other ratios. On the contrary, the specification teaches that a very wide ratio range between 0.005:1 and 25:1 can be used in the instant method and the range of 0.01 taught by Yang et al. definitely falls within the range taught by the specification (see the specification, p. 1, paragraph 0009, p. 7, paragraphs 0102). The only teaching in the specification regarding the enzyme amount is that increasing the amount of RNase III results in increased yields for both magnesium and manganese, which was already known in the prior art; the difference is that yields obtained with manganese are higher than yields obtained with magnesium (see the specification, p. 7, paragraph 0102), which is taught by the combination of Yang et al. and Gross et al. (see the rejection above). Therefore, absent evidence of unexpected results, one of skill in the art would have known to use routine experimentation to optimize the results (see MPEP 2144.05 [R-3] II, Obviousness of Ranges). It is noted that Applicant only amended claim 1 to include the ratio of 0.25:1 to overcome the 102(e) rejection over Yang et al.

With respect to the method yielding overlapping fragments, Applicant argues that the discovery that overlapping fragments could be produced was Applicant's discovery and was not evidenced in the art on which the rejection relies. Applicant argues that Yang et al. do not demonstrate the production of overlapping fragments and that, based on Gross et al. suggesting that, at least in one context, RNase III-mediated cleavage

happens at defined positions, one of skill in the art would not expect that cleavage products would be overlapping. It is noted that Gross et al. teach a 141 nucleotides long RNA, which contains short double-stranded RNA regions separated by regions of single-stranded RNA, wherein the short double-stranded RNA regions contain the specific sites for RNase III (see Gross et al., Abstract, p. 431, p. 438, Fig. 3). Since the double-stranded RNA regions are short and separated by ling single-stranded regions, one of skill in the art would not expect to obtain overlapping fragments when using such an RNA. However, the combined Yang et al. and Gross et al. teach a totally different RNA, i.e., a long double-stranded RNA having a multitude of RNase III cleavage site, wherein the RNase III randomly cleaves the RNA to yield overlapping fragments. Moreover, it is not clear why the same *E. coli* RNase III would be able to produce overlapping fragments when used in the instant method and not be able to do so in the method taught by Yang et al. and Gross et al., which is similar to the instant method; the method of Yang et al. and Gross et al. would necessarily result in overlapping fragments representing a substantial portion of the RNA from which they are derived, because the RNase III sites are spread over the entire lengths of the RNAs. Therefore, Applicant only observed an inherent property of RNase III and this cannot be considered an unexpected result.

With respect to the combination of references, Applicant argues that the two references teach away from each other, because Yang et al. teach limiting the digestion, while Gross et al. teach that additional sites are cut in the presence of manganese; therefore, one of skill in the art would not want to exacerbate the problems

of Yang et al. and would not have been motivated to modify Yang et al. according to the teachings of Gross et al. In response to this argument it is noted that Gross et al. teach that, while in the presence of manganese additional secondary sites are exposed, a complete digestion of double-stranded RNA cannot be achieved; Gross et al. teach that, after cleavage at a particular secondary site, the RNA refolds so that the cleavage at other secondary sites is difficult if not impossible (see Gross, p. 441). Based on these teachings, one of skill in the art would have known that manganese improves the cleaving efficiency of RNase III without over-digesting the RNA and would not conclude that Gross et al. teach away from Yang et al. One of skill in the art would have been motivated to use manganese in the method of Yang et al. to improve reaction efficiency, by exposing some of the secondary sites in the RNA; based on the teachings of Gross et al., one of skill in the art would have known that incorporation of manganese would not cleave the double-stranded RNA into fragments too small to be of interest.

With respect to the significant advantages of the claimed invention, it is noted that claim 1 does not recite limitations such as generating fragments less than 50 bases without degrading the fragments, using the fragments without size fractionation, or that a substantial portion of the substrate sequence is represented by the fragments. And even if they were, it is noted that the method of Yang et al. and Gross et al. would have necessarily resulted in fragments 15-30 bases that could no longer be digested (see the teachings of Gross above), wherein the fragments represent a substantial portion of the substrate RNA, because the cleavage sites are evenly distributed along the RNA and because both methods use the same enzyme that nonspecifically cuts the RNA (see

Yang et al., paragraph 0053). Along these lines, Yang et al. teach that their method is capable of generating a great variety of fragments capable of interacting with multiple sites on the target RNA (p. 2, paragraph 0013).

Therefore, the claimed method does involve the predictable use of prior art elements according to their known function, i.e., manganese increases the efficiency of reaction while limiting over-digestion.

B. Claims 2 and 9

Applicant argues that Yang et al. teach away from complete digestion, as recited in claims 2 and 9. It is noted that Yang et al. only caution against exhaustive digestion (see p. 2, paragraph 0015). By teaching limited digestion, Yang et al. do not necessarily teach that complete digestion to fragments of 15-30 bp cannot be achieved; on the contrary, as noted above, Yang et al. teach conditions of limited digestion (i.e., 15 min at 37°C) wherein fragments of 15-30 bases are produced. Therefore, it is clear that Yang et al. teach limited digestion conditions that result in the accumulation of 15-30 bases long fragments, conditions that do not result in exhaustive digestion.

C. Claims 5-7

Applicant argues that one of skill in the art would not have been motivated to use manganese, as recited in claims 5-7, because Gross et al. teach that manganese promotes cleavage at additional sites. The response to this argument is the same as set forth above for claim 1.

D. Claim 12

Applicant argues that one of skill in the art would not have reasonably expected that a digestion according to Yang et al. and Gross et al. would lead to products that could be used in a method of silencing, since exhaustive digestion would result in fragments too small to trigger interference. The response to this argument is the same as set forth above for claim 1.

E. Claims 13, 14, 16-18, 20, and 47

Applicant argues that neither Yang et al. not Gross et al. teach fragments that are overlapping and represent a substantial portion of the sequence of the target RNA, as required by claims 13, 14, 16-18, 20, and 47. The response to this argument is the same as set forth above for claim 1.

(11) Related Proceeding(s) Appendix

No decision rendered by a court or the Board is identified by the Examiner in the Related Appeals and Interferences section of this Examiner's answer.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

Ileana Popa, PhD

Conferees:

/Joseph Woitach/

Joseph Woitach

SPE 1633 Joseph Woitach

RAM R. SHUKLA, PH.D.
SUPERVISORY PATENT EXAMINER

Ram Shukla

SPE 1634

RAM R. SHUKLA, PH.D.
SUPERVISORY PATENT EXAMINER